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Antibacterial activities of *Camellia sinensis* plant extracts against uropathogenic *E. coli in vitro* and *in vivo*

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This study evaluated the *in vitro* and *in vivo* antibacterial activities of methanol and ethyl acetate extracts of *Camellia sinensis* (Green tea) against *Escherichia coli* implicated in urinary tract infections. Antibacterial activities of these plant extracts were tested against 10 multi-drug resistant *E. coli* isolates obtained from clinical specimens of UTI patients. Forty-five (45) Wister albino rats were used for this study and exactly 0.1 ml of standardized (0.5 McFarland's) *E. coli* suspension was each inoculated into the 45 rats through intra-urethral route and observed after 48 h. This was followed by oral administration of different concentrations of methanol and ethyl acetate extracts of *C. sinensis*, and ciprofloxacin antibiotic for 14 days. Phytochemical screening of extracts showed the presence of bioactive components. Results revealed that methanol extract was better than ethyl acetate extract of *C. sinensis* in the treatment of UTI caused by *E. coli*. Body weight, white blood cell count, and erythrocyte sedimentation rate level returned to normal values after treatment with methanol extract of *C. sinensis* at 200 mg/kg body weight when compared to ethyl acetate extract of *C. sinensis*. This study has shown that *C. sinensis* possess bioactive ingredients with antimicrobial activities. Information from this study adds to the current information on the potential health benefits of green tea. Thus, further studies on other plant products should be explored so as to understand their potential health benefits and as alternative therapeutics in the treatment of bacterial infections.

Key words: Antibacterial activity, *E. coli*, *Camellia sinensis*, urinary tract infections (UTIs), plant extract

INTRODUCTION

Antimicrobial agents (β-lactams, fluoroquinolones, trimethoprim–sulfamethoxazole, nitrofurantoin, etc.) that have traditionally been used to treat UTIs are becoming less effective due to the increasing frequency of

antibiotic-resistant bacteria (Zhanel et al., 2006). As a result, there is need to search for alternative to these drugs. Green tea is derived from non-fermented leaves of *Camellia sinensis* plant. Oolong and black tea are made from fermented leaves of the same plant. Traditionally, green tea has been a favoured drink in Asian countries because of its health benefits. Green tea, as recalled in several ways, is now gaining worldwide popularity as an important drink in preventative medicine (Friedman, 2007). Urinary tract infection (UTI) is a disease involving the kidneys, ureters, bladder, or urethra. It is the second most common type of infection found in any organ/system, and the most common type of nosocomial infection (Carson and Naber, 2004). The strains of *E. coli* that infect the urinary tract are categorized as uropathogenic *Escherichia coli* (UPEC) (Kaper et al., 2004).

The UPEC are able to produce special surface proteins (adhesins) that allow them to attach and invade epithelial cells that line the urinary bladder (Marrs et al., 2005). If the infection is not treated while it is in the bladder (uncomplicated UTI), some strains of UPEC may then travel up the ureters to the kidneys and cause even more severe infections, leading to complicated UTIs, with consequences of renal damage and possibly renal failure (Pichon et al., 2009). Prior to the discovery of antibiotics, herbal medicine was known and used to treat various infections including UTIs. Some of the herbs which have antibacterial activities are *Allium sativum*, *Zingiber officinale*, *Cymbopogon citrates*, *Murraya koenigii*, and *C. sinensis* (source of green tea). Roccaro et al. (2004) reported that properties of green tea which inhibit bacterial growth are as a result of their polyphenols, mainly the flavonoids especially catechins. Vuong et al. (2011) also reported that green tea extract has been very effective in treating UTIs. Hsu et al. (2003) reported that the highest antimicrobial activity of green tea is due to the presence of catechins and polyphones which damage bacterial cell membrane. This study was therefore designed, to determine the *in vitro* and *in vivo* antibacterial activities of methanol and ethyl acetate extracts of *C. sinensis* (Green tea) against *E. coli* implicated in urinary tract infections.

MATERIALS AND METHODS

Collection of biological materials

A total of 10 clinical isolates of *E. coli* from UTI patients were collected from the Microbiology Laboratory Unit of Federal Teaching Hospital Abakaliki (FETHA II), while medicinal plant materials used were commercially available powdered (Oriba) *C. sinensis* (Green tea) purchased from Yaoundé, Cameroun.

Re-confirmation of bacterial isolates

Isolates were re-confirmed using standard microbiological techniques. Isolates were firstly sub-cultured on MacConkey agar (Oxoid, UK) and incubated for 24 h at 37°C. After incubation, pink colonies of *E. coli* were then sub-cultured on nutrient agar and incubated for 24 h at 37°C. Discrete colonies of *E. coli* were then purified through successive streaking on nutrient agar. Stock culture was maintained at 4°C on nutrient agar slants. Active cultures for experiments were prepared by transferring a loopful of the test organism (*E. coli*) to nutrient agar plate using a sterile wire loop and incubated at 37°C for 24 h (Jayaraman et al., 2008; Cheesbrough, 2004).

Preparation of ethyl acetate and methanol extracts of *Camellia sinensis*

Twenty (20) grams of powdered (Oriba) *C. sinensis* (Green tea) was weighed and soaked in 200 ml of ethyl acetate and methanol in conical flasks and covered with an aluminum foil. The mixtures were stirred and agitated on the shaker for 24 h. After then, it was filtered using muslin cloth, air dried, and stored at 4°C in airtight bottles for further studies (Atata et al., 2003; Mbata et al., 2008).

Preparation of *Camellia sinensis* stock solution

Three (3) gram of dried *C. sinensis* extract was weighed and dissolved in 5 ml of Dimethyl sulphoxide (DMSO) solution. Thus, 200 mg/ml of stock solution was obtained as standard concentration of extract (Almola, 2010).

Crude extract preparation

Powdered *C. sinensis* was weighed and soaked in methanol in ratio 6:1 (methanol: plant) for 2 days and filtered using Whatman No. 1 filter paper. The methanol was completely removed by vacuum evaporator at 50°C until it produces a viscous mass. The crude extracts were weighed and stored at 4°C before analysis (Tariq and Reyaz, 2012).

Antimicrobial sensitivity test (*in vitro* testing)

Antibiotic susceptibility test was determined using the Kirby-Bauer disc diffusion method according to the recommendations of the Clinical and Laboratory Standard Institute - CLSI (CLSI, 2015). The isolates were sub-cultured on nutrient agar, and incubated at 37°C for 18-24 h. Thereafter, the colonies of each of the isolate were adjusted to 0.5 McFarland turbidity standards in sterile nutrient broth. The standardized broth culture was incubated for 10 min and using sterile swab stick, the standardized broth culture of the isolates was inoculated onto Mueller-Hinton agar plates. The surface of the medium was streaked in four directions while the plates were rotated approximately 60° to ensure even distribution. The inoculated Mueller-Hinton agar plates were allowed to dry for few minutes. The following standard antibiotic discs (Oxoid, UK) were used against the isolates; ciprofloxacin, aztreonam, nalixidic

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acid, ceftazidime, and cefotaxime. Sterilized forceps were used to place the antibiotic discs evenly on the inoculated Mueller-Hinton agar so that the disc should be about 15 mm from the edge of the plate and not closer than 25 mm from disc to disc. After 30 min, the plates were inverted and incubated for 24 h. A ruler was used to measure the diameter of each zone of inhibition in mm on the underside of the plate. The inhibitory zone diameter was interpreted as susceptible or resistant according to the criteria of CLSI (2015).

Determination of antibacterial activity of *Camellia sinensis* extracts

The surface of Mueller-Hinton agar plates was streaked with standardized inoculums of the test bacteria that have been adjusted to 0.5 McFarland's turbidity standards. A sterile cork borer of 8 mm diameter was used to bore holes (2) on each of the Mueller-Hinton agar plates, and the holes were filled with equal volumes of plant extracts that were diluted with DMSO. The plates were allowed to stand on the bench for proper diffusion within an hour, and after then, incubated at 37°C for 24 h. The resulting inhibition zones diameter was measured in millimeters (mm) using a meter rule. Ciprofloxacin (5 mg/ml) and Nalidixic acid were used as positive control antibiotics (Esimone et al., 2008).

Determination of minimum inhibitory concentration (MIC) of *Camellia sinensis* extract

The MIC of *C. sinensis* extract was determined using agar well diffusion technique. From the stock of 200 mg/ml extract, five-fold serial dilution was made to 100, 50, 25, 12.25, 6.25, and 3.25 mg/ml (CLSI, 2015). The surface of solidified sterile Mueller-Hinton agar plates was inoculated with 0.5 McFarland's standardized test bacteria. A cork borer of 8 mm was used to bore holes on the seeded Mueller-Hinton agar. The *C. sinensis* extract of different concentration was used to fill the holes. The test plates were incubated at 37°C for 24 h. MIC was defined as the lowest concentration of extract inhibiting bacterial growth (EUCAST, 2000).

Experimental design

Forty-five (45) Wister albino rats were divided equally into nine groups; five rats in each group (treatment began after 48 h of inducing infection). Group A: normal control (not infected group was given only distilled water orally for 14 days); Group B: Negative control (infected and not treated group); Group C: positive control (infected and treated with ciprofloxacin orally for 14 days). Group D_1: infected with *E. coli* and treated orally with 200 mg/kg of ethyl acetate extract of *C. sinensis* for 14 days; Group D_2: infected with *E. coli* and treated orally with 400 mg/kg of ethyl acetate extract of *C. sinensis* for 14 days; Group D_3: infected with *E. coli* and treated orally with 600 mg/kg of ethyl acetate extract of *C. sinensis* for 14 days; Group E_1: infected with *E. coli* and treated orally with 200 mg/kg of methanol extract of *C. sinensis* for 14 days; Group E_2: infected with *E. coli* and treated orally with 400 mg/kg of methanol extract of *C. sinensis* for 14 days; and Group E_3: infected with *E. coli* and treated with 600mg/kg of methanol extract of *C. sinensis* for 14 days (Debiao et al., 2008).

Clinical signs

Animals were observed continuously for clinical signs; color of urine, unusual frequency in urination and foul smelling or cloudy urine in infected groups. Changes in activity, behaviour, and death rate of the animals were also recorded weekly throughout the experimental period.

Body weight changes

The weight of the animals was measured and differences were assessed according to feed requirement and comparison between treated and control animals. These measurements were done during the first week-before inducing infection, and after 7 and 14 days post treatments.

Preliminary phytochemical screening

This was carried out to determine the active phytochemical constituents present in *C. sinensis* such as alkaloids, glycosides, terpenoids, steroids, flavonoids, saponin, and tannin (Zuo et al., 2002).

Alkaloid determination: The methanol crude extract was heated on a boiling water bath with 2% hydrochloric acid. After cooling, the mixture was filtered and treated with a few drops of 5% sodium hydroxide solution. The filtrate was observed for the presence of turbidity or yellow precipitation.

Glycoside determination: Exactly 0.5 g of extract was dissolved in 2 ml of glacial acetic acid and properly mixed. Two drops of ferric chloride and concentrated sulphuric acid were added and observed for a reddish brown coloration at the junction of two layers and bluish-green color in the upper layer.

Terpenoid determination: A total of 4 μg of extract was treated with 0.5 ml of acetic anhydride and 0.5 ml of chloroform. Concentrated solution of sulphuric acid was then added slowly and red violet color was observed for the presence of terpenoid.

Steroid determination: Four (4) μg of the extract was treated with 0.5 ml of acetic anhydride and 0.5 ml of chloroform. Thereafter, concentrated solution of sulphuric acid was added slowly and green-bluish colour indicated presence of steroids.

Flavonoid determination: Two (2) ml of extract solution was treated with 1 ml of lead acetate solution and white colour indicated the presence of flavonoids.

Saponin determination: Exactly 0.5 μg of extract was treated with 5 ml of distilled water. Frothing persistence indicated the presence of saponins.

Catecholic tannin determination: Exactly 0.5 μg of extract was dissolved in 1 ml of water and mixed uniformly. A total of 2 drops of ferric chloride solution was then added to the mixture and observed for green black colour, indicating the presence of catecholic tannin.

Gallic tannin determination: Exactly 0.5 μg of extract was dissolved in 1 ml of water and mixed uniformly. Then, 2 drops of ferric chloride solution were added and observed for blue coloration, indicating the presence of gallic tannin.

Re-isolation of uropathogenic *E. coli*

Re-isolation of uropathogenic *E. coli* was performed with the urine specimens that were obtained with sterile swabs according to the technique of Kurien et al. (2004). This procedure involves holding the rat over a swab (hold the swab close to urinary outlet) and encouraging it to micturate. Fresh, clean sample for the analysis of the entire urinary tract was obtained by expression of the bladder manually onto the swab. *E. coli* counts in urine samples were performed before infection, 2 days after infection, and at the end of 7 and 14 days of treatment.

Table 1. Phytochemical screening of green tea *(Camellia sinensis).*

S/N	Phytochemical constituent	Intensity
1	Saponins	+
2	Flavonoids	+
3	Terpenoids	+
4	Glycosides	+
5	Alkaloids	+
6	Phenols	+
7	Tannins	+
8	Steriods	-

+ = Present, - = Absent.

Haematological analysis

Haematology test include laboratory assessments of blood formulation and blood disorders. Haematological indices of the rats were studied to include the values of the cellular elements of blood like erythrocyte sedimentation rate and white blood cell counts using Westergren tube or wintrobe tube and haemocytometer (Improved Neubauer) respectively (Cheesbrough, 2004).

Erythrocyte sedimentation rate (ESR)

When an anticoagulant was added to the blood and well-mixed venous blood was placed in a vertical tube, erythrocytes tend to settle towards the bottom leaving clear plasma on top. This rate of sedimentation of red blood cells in a given interval of time is called erythrocyte sedimentation rate (ESR). Four (4) ml of venous blood was withdrawn and mixed with 10 ml of sodium citrate. The mixture in the tubes were inverted 2-3 times to mix the blood thoroughly with anticoagulant .The Westergren tube were filled up to mark 0, placed in the rack at room temperature undisturbed, and away from sunlight. Readings were taken after 1 h and recorded in millimeters (mm) from the top surface of the column to the top of erythrocyte sediments (Cheesbrough, 2004).

White Blood Cell Count (WBC)

Whole blood collected in EDTA was diluted according to the type of cell count obtained. The diluted blood suspension were then placed in a chamber and the cell counted. Counted cells were multiplied by their dilution factors and reported as the number of cells per microlitre (µl) of whole blood. The top of grid was covered with cover slip/glass slip within the chamber (air tight technique). The samples were diluted in the ratio of 1:20 (acetic acid: whole blood) for WBC count. Samples were loaded into the loading area in the chamber, and cells were counted in the 4 large squares for WBC (Cheesbrough, 2004).

Statistical analysis

Data was analyzed using Statistical Package for Social Sciences (SPSS) software version 17.0 version. Statistical analysis of data was performed by Two-Way Analysis of Variance (ANOVA). Specific group differences were determined using least significant differences (LSD). Results will be considered statistically significant if the p value is less than 0.05 ($p < 0.05$).

RESULTS

The phytochemical analysis of green tea extracts showed the presence of saponins, flavonoids, terpenoids, glycosides, alkaloids, phenonls, and tannins while steroids were absent (Table 1).

Ciprofloxacin was the most effective antibiotic tested against the isolates as 90% of the isolates were susceptible. Exactly 50, 20, and 10% of the isolates were susceptible to nalidixic acid, cefotaxime, and aztreonam respectively while all the isolates were resistant to ceftazidime (Table 2).

The result of antibacterial activity of methanol and ethyl acetate extracts of *C. sinensis* on isolates at 200 mg/ml is shown in Table 3. Ethyl acetate extract were ineffective against isolates 3, 4, and 5 while it was very effective against isolate 1 with the highest inhibition zone diameter (IZD) of 20 mm. Methanol extract of *C. sinensis* showed its highest activity against isolate 9 with IZD of 22 mm. Methanol extract also showed activity against isolates 5, 7, and 10 with IZD of 21 mm each while IZD of 20 mm was each observed against isolates 3, 6, and 8 (Table 3).

Result of minimum inhibitory concentration (MIC) of methanol extracts of *C. sinensis* against isolates is presented in Table 4. The MIC of methanol extracts of *C. sinensis* was 3.25 mg/ml against isolates 1, 6, 7, 8, 9, and 10 while it was 6.25 and 100 mg/ml against isolates 2 and 3 respectively. MIC value of 50 mg/ml was each recorded for isolates 4 and 5 (Table 4).

The minimum inhibitory concentration (MIC) of ethyl acetate extract of *C. sinensis* was 3.25 mg/ml against *E. coli* isolates 6, 7, 8, and 9 (Table 5). MIC value of 12.25 mg/ml was recorded against isolate 10 while MIC value of 50 mg/ml was each recorded against isolates 1, 2, and 6 (Table 5). The changes in body weight gave an evidence of correlation between infection, the kind of treatment and the dose of treatment that were used in different groups (Table 6). After 48 h of infection and 14 days of treatment, there was a significant increase ($P < 0.05$) in body weight of all infected and treated groups when compared with group A (normal control). After 14 days of treatment, the group treated with methanol extract, ethyl acetate extract, and ciprofloxacin (500 mg/kg) antibiotic showed weight gain after 14 days of treatment when compared with the non-treated groups (Table 6).

All healthy animals before induction of infection presented normal urine with yellow colour. Forty-eight hours after induction of infection in experiment animals, animals suffered from anorexia; dehydration, fever, and their urine were dark yellow. Unusual frequency of urination increased gradually from the first day after infection with foul smelling and cloudy urine in infected groups. Rats in group A (normal control) showed no significance increase in bacteria count before and after 14 days of treatment, while those in group B (negative control) showed significant increase ($P < 0.05$) in bacteria count after 48 h of induction of infection. The group E (induced and treated with methanol extract) showed great significant increase in bacteria count ($P < 0.05$) after 48 h of induction, but showed significant decrease ($P < 0.05$) in bacteria count after 7 to 14 days of treatment,

Table 2. Antibiotic sensitivity pattern of isolates.

Sample	NA	CRO	ATM	CAZ	CXM
Isolate 1	R	S	R	R	R
Isolate 2	R	S	R	R	R
Isolate 3	S	R	R	R	R
Isolate 4	R	S	R	R	R
Isolate 5	R	S	R	R	R
Isolate 6	R	S	R	R	R
Isolate 7	S	S	R	R	R
Isolate 8	S	S	R	R	S
Isolate 9	S	S	R	R	R
Isolate 10	S	S	S	R	S
Total susceptibility (S) frequency (%)	50	90	10	0	20

NA = Nalidixic acid, CRO = Ciprofloxacin, ATM = Aztreonam, CAZ = Ceftazidime, CXM = cefotaxime, S = Susceptible, R = Resistant.

Table 3. Antibacterial activity of methanol and ethyl acetate extract of *Camelia sinensis* against test isolates at 200 mg/ml.

Sample	Methanol	Ethyl acetate	NA	CRO
	Inhibition Zone Diameter (mm)			
Isolate 1	19	20	NI	23
Isolate 2	17	17	NI	25
Isolate 3	20	NI	10	NI
Isolate 4	19	NI	NI	20
Isolate 5	21	NI	NI	27
Isolate 6	20	16	NI	17
Isolate 7	21	15	20	10
Isolate 8	20	17	23	30
Isolate 9	22	18	15	13
Isolate 10	21	18	22	30

NI = No inhibition, NA = Nalidixic acid, CRO = Ciprofloxacin.

Table 4. Minimum inhibitory concentration of methanol extract of *Camellia sinensis* against test isolates.

S/No.	Sample	MIC (mg/ml)	IZD (mm)
1	Isolate 1	3.25	25
2	Isolate 2	6.25	20
3	Isolate 3	100	20
4	Isolate 4	50	13
5	Isolate 5	50	15
6	Isolate 6	3.25	18
7	Isolate 7	3.25	14
8	Isolate 8	3.25	17
9	Isolate 9	3.25	20
10	Isolate 10	3.25	16

MIC = Minimum inhibitory concentration, IZD = Inhibition zone diameter.

especially in the group that was treated with 200 mg/kg of methanol extract when compared to group C (positive control). Group D which was treated with ethyl acetate extract showed significant increase ($P < 0.05$) in bacteria count after 48 h of induction, but after 7 to 14 days of treatment, there was significant decrease ($P < 0.05$) in

Table 5. Minimum inhibitory concentration of ethyl acetate extracts of *Camellia sinensis* on *E. coli* isolates.

Sample	MIC (mg/ml)	IZD (mm)
Isolate 1	50	15
Isolate 2	50	15
Isolate 3	NI	NI
Isolate 4	NI	NI
Isolate 5	NI	NI
Isolate 6	3.25	14
Isolate 7	3.25	15
Isolate 8	3.25	20
Isolate 9	3.25	17
Isolate 10	12.25	14

MIC = Minimum inhibitory concentration, IZD = Inhibition zone diameter, NI = No inhibition.

Table 6. Weight (in grams) of rats infected with *E. coli* and treated with *Camellia sienensis.*

Weight / Group	Weight before treatment (g)	Weight after treatment (g)
Group A	207.68 ± 33.61[a]	228.58 ± 29.68[b]
Group B	212.84 ± 35.10[a]	192.58 ±35.28[a]
Group C	207.8 ± 47.59[a]	212.68 ± 33.9[a]
Group D1	189.94 ± 15.33[a]	210.92 ±18.18[a]
Group D2	202.64 ± 18.3[a]	216.48 ± 24.65[a]
Group D3	191.08 ± 17.32[a]	203.06 ± 29.07[a]
Group E1	213.72 ± 27.59[a]	208.7 ± 36.68[a]
Group E2	201.88 ± 12.06[a]	217.44 ± 22.75[a]
Group E3	231.96 ± 32.81[a]	234.9 ± 25.56[b]

Group–A (Normal Control); Group-B (Negative Control: Induce without Treatment); Group-C (Positive Control: Induced and Treated with Standard drug); Group-D1 (Induced and treated with 200 mg/kg of ethyl acetate); Group-D2 (Induced and treated with 400 mg/kg of ethyl acetate); Group-D3 (Induced and treated with 600 mg/kg of ethyl acetate); Group-E1 (Induced and treated with 200 mg/kg of methanol extract); Group-E2 (Induced and treated with 400 mg/kg of methanol extract); Group-E3 (Induced and treated with 600 mg/kg of methanol extract).

bacteria count especially for the group that was treated with 600 mg/kg of ethyl acetate extract (Table 7). After 7 to 14 days of treatment, the symptom of disease, such as strong and frequent urge to urinate, cloudy, bloody, and strong-smelling urine reduced in treated rats and the total White Blood Cell (WBC) count decreased significantly ($P < 0.05$) from 6.25 ± 0.07^a to 2.85 ± 0.64^a. There was no significant difference between erythrocyte sedimentation rates of control groups, infected, and treated rats (Table 8).

DISCUSSION

Kumar et al. (2006) reports have shown that different plants, herbs, and spices contain many natural occurring compounds that possess antimicrobial function and serve as antimicrobial substance against clinically isolated bacteria. Majority of mortality and morbidity rate worldwide are caused by bacterial infectious diseases therefore, there is an increasing interest in the development of new antibacterial substances for the treatment of infectious diseases caused by bacteria. Our study evaluated the *in vitro* and *in vivo* antibacterial activities of methanol and ethyl acetate extracts of *C. sinensis* (Green tea) against *E. coli* implicated in urinary tract infections. This study showed that green tea possesses bioactive compounds such as flavonoids, saponins, terpenoides, glycosides, alkaliodes, and tannins which have antimicrobial activities. Results of this study revealed that methanol extract was better than ethyl acetate extract of *C. sinensis* in the treatment of UTI caused by *E. coli*. This study also observed that the body weight, white blood cell count, and erythrocyte sedimentation rate level in infected rats returned to normal values after treatment with methanol extract of *C. sinensis* at 200 mg/kg body weight, when compared to ethyl acetate extract of *C. sinensis*, and 500 mg/kg of ciprofloxacin antibiotic. The

Table 7. Urine bacteria count in cfu/ml in rats infected with *E. coli* and treated with *Camellia sinensis* (Green Tea).

Group	Bacteria count before induction	Bacteria count after 48 h of induction	Bacteria count after 7 days of treatment	Bacteria count after 14 days of treatment
Group A	93.8±53.14^{ab}	93.6±53.87^{a}	94.2±53.87^{a}	94.4±53.39^{a}
Group B	71.2±30.12^{ab}	96.8±10.06^{a}	106.4±9.74^{a}	118.6±13.18^{a}
Group C	73.60 ± 23.43^{ab}	162.80 ±84.23^{ab}	23.6000 ± 6.07^{b}	10.60 ± 2.70^{b}
Group D1`	75.200 ± 48.28^{ab}	139.20 ± 69.822^{ab}	41.200 ± 14.81^{b}	26.00 ± 13.60^{b}
Group D2	121.20 ± 34.40^{b}	194.40 ± 100.97^{b}	26.60 ± 9. 10^{b}	18.60 ± 9.92^{b}
Group D3	87.20 ± 39.63^{ab}	153.60 ± 36.94^{ab}	35.40 ± 38.90^{b}	11.60 ± 7.16^{b}
Group E1	53.60 ± 37.27^{a}	104.80 ± 29.85^{a}	21.60 ± 11.17^{b}	10.80 ± 1.10^{b}
Group E2	56.80 ± 34.57^{a}	110.40 ± 31.19^{a}	30.40 ± 25.86^{b}	13.00 ± 5.61^{b}
Group E3	61.20 ± 13.83^{a}	82. 40 ± 20.12^{a}	25.60 ± 11. 87^{b}	13.60 ± 6.69^{b}

Group–A (Normal control); Group-B (Negative control: Induced without treatment); Group-C (Positive control: Induced and treated with standard drug); Group-D1 (Induced and treated with 200 mg/kg of Ethyl acetate); Group-D2 (Induced and treated with 400 mg/kg of ethyl acetate); Group-D3 (Induced and treated with 600 mg/kg of ethyl acetate); Group-E1 (Induced and treated with 200 mg/kg of methanol extract); Group-E2 (Induced and treated with 400 mg/kg of methanol extract); Group-E3 (Induced and treated with 600 mg/kg of methanol extract).

Table 8. White blood cell (WBC) count and erythrocyte sedimentation rate (ESR) of rats infected with *E. coli* and treated with *Camellia sinensis.*

Group	WBC (µl)	ESR (mm/h)
Group A	11.95 ±11.82^{a}	1.00 ±0.00^{a}
Group B	6.25 ± 0.07^{a}	1.00 ± 0.00^{a}
Group C	2.85 ± 0.64^{a}	1.50 ±0.00^{a}
Group D1`	6.00 ± 0.00^{a}	1.00 ± 0.00^{c}
Group D2	6.90±0.00^{a}	1.00±0.00ab^{c}
Group D3	4.60 ±0.00^{a}	1.00 ± 0. 00^{a}
Group E1	3.00 ± 0. 00^{a}	3.00 ± 0. 00^{a}
Group E2	5.60 ± 0.00^{a}	1.00 ± 0.00a^{bc}
Group E3	6.10 ±0.00^{a}	1.00 ± 0. 00^{bc}

Group – A (Normal control); Group- B (Negative control: Induced without treatment); Group-C (Positive control: Induced and treated with standard drug); Group-D1 (Induced and treated with 200 mg/kg of ethyl acetate); Group-D2 (Induced and treated with 400 mg/kg of ethyl acetate); Group-D3 (Induced and treated with 600 mg/kg of ethyl acetate); Group-E1 (Induced and treated with 200 mg/kg of methanol extract); Group-E2 (Induced and treated with 400 mg/kg of methanol extract); Group-E3 (Induced and treated with 600 mg/kg of methanol extract).

presence of phytochemical compounds such as alkaloids, flavonoids, tannins, saponins, terpenoids, phenonls, and glycosides indicated that the extracts of *C. sinensis* possess antimicrobial activities; and this confirmed its uses against bacterial infections. In this study, *C. sinensis* contains alkaloids, flavonoids, saponins, terpenoids, glycosides, steroids, and tannins. This work agrees with the work of Hsu et al*.* (2003) who reported that the highest antimicrobial activity of tea is due to presence of catechins and polyphones which damage bacterial cell membrane. They also serve in plant defense mechanisms to counteract reactive oxygen species in order to survive and prevent molecular damage caused by microorganisms, insects, and herbivores (Akowuah et al., 2005). Tannins have also been reported to possess strong, broad spectrum antibacterial properties (Amarowicz et al., 2008; Min et al., 2008; Doss et al., 2009). The properties of green tea which inhibit bacterial growth are owing to their polyphenols, mainly the flavonoids especially catechins (Roccaro et al., 2004). Our study showed that methanol extract of green tea exhibited greater antimicrobial activity, producing larger inhibition zone diameter (IZD) against uropathogenic *E. coli* isolates. These observations may be attributed to established reports that bioactive components in green tea such as catechin compounds and polyphenols possess antibacterial activity. The methanol extracts of *C. sinensis* exhibited the highest antibacterial activity against *E. coli* with IZD of 22 mm while *E. coli* was resistant to ciprofloxacin and nalidixic acid. This result agrees with the study of Shahidi (2004), who reported that methanol extract of *C. sinensis* (20 mg/ml) had antibacterial activity against one strain of *E. coli* (PTCC No. 1338) with IZD of 10 mm. The report by Shahidi

(2004) suggested that green tea can be used in the treatment of bacterial infections caused by *E. coli* that was initially resistant to antibiotics such as trimethoprim-sulfamethoxazole. Our study also agrees with the work of Bandyopadhy et al. (2005), who reported that green tea and black tea exhibited antimicrobial activity against multidrug-resistant *E. coli* ATCC 25922. This study showed that methanol extract of *C. sinensis* (green tea) were active (IZD of 21 mm) against uropathogenic *E*. *coli*. This is in accordance with the work of Saikia et al. (2006), who reported *E. coli, Enterococcus faecalis*, *Staphylococcus aureus*, *Candida albicans*, and *Pseudomonas aeruginosa* were susceptible to methanolic extract of green tea. The ethyl acetate extract of *C. sinensis* showed the highest activity against *E. coli* isolate 1with IZD of 20 mm, followed by IZD of 18 mm each against *E. coli* isolates 9 and 10. This report is in concord with that of Chan et al. (2011) who reported that aqueous extracts of green tea proved effective against a variety of Gram-positive and Gram-negative bacteria using similar extraction techniques. In this study the green tea catechins did not work synergistically with ciprofloxacin or nalidixic acid against any of the test samples. The zone of inhibition was not greater than the sum of both antibacterial agents. Our study has shown the effectiveness of *C. sinensis* in the treatment of urinary tract infection and this result is similar to other studies which reported that green tea extract has inhibitory effects on the growth of *E. coli* strains isolated from UTIs (Vuong et al., 2011). Studies have shown that concentrations of 500 µg of tea polyphenols can inhibit the growth of *E. coli*, and that concentration of ≥ 5000 µg are considered bactericidal. This effect is believed to be due to the fact that tea polyphenols down regulate the production of proteins such as EF-2 (elongation factor for protein translation) proteins involved in phospholipid, carbon, and energy metabolism; and production of proteins involved in amino acid biosynthesis (Cho et al., 2007). In our study, urine test results after inoculation showed significant increase ($P > 0.05$) in *E*. *coli* when compared to the test results prior to inoculation, thus indicating apparent presence of bacterial infection. There was a significant decrease ($P < 0.05$) in the colony forming units (CFU) of bacteria in urine of all the induced and treated rats during the treatment process. The observed reduction in colony counts of bacteria in urine of infected and treated rats in our study prove the efficacy of *C. sinensis* extracts in the treatment of UTIs. Our study showed that there was no significant difference between erythrocyte sedimentation rates of control rat groups, infected groups, and treated groups.

Conclusion

This study has shown that methanol and ethyl acetate extracts of *C. sinensis* possess antibacterial activity against uropathogenic *E. coli* causing urinary tract infections (UTIs). Our study also showed that *C. senensis* contains bioactive ingredients such as flavonoids, tannins, saponin, terpenoids, phenols, alkaloids and glycosides. Interestingly, this study revealed that methanol extract of *C. sinensis* (green tea) exhibited higher antibacterial activity against the test uropathogenic *E. coli* isolates at lower concentration than ethyl acetate extract. Data from this study also adds to the current information on the potential health benefits of green tea. It is hopeful that these findings will encourage further studies on the antimicrobial potentials of commercially prepared green tea and other plant products such as black tea and coffee.

CONFLICT OF INTERESTS

The authors have not declared any conflicts of interest.

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